FACTORS AFFECTING TERMINAL GROWTH IN THE HYDROID CAMPANULARIA [1]

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The experiments reported here pertain to the limitation of terminal growth in the hydroid *Campanularia flexuosa*. The general problem of growth limitation, and specifically of terminal growth, has been examined for many different organisms. A review is impossible in a brief paper. Few general unifying concepts seem to have been established clearly: we are still in the stage of assembling the necessary facts.

Three considerations led to these particular experiments. First, the fact that each species of hydroid has a stem of characteristic and fairly definite height suggests the presence of limiting factors which must either vary from species to species or have a variable effectiveness. Secondly, an earlier experiment (Crowell, 1957) had shown that terminal growth is very sensitive to nutritional level, and also that the effects of lowered nutrition are more striking the older the stem. This assured us that the terminal growth zone is not autonomous but has a dependence upon events and conditions beyond itself. Thirdly, there was a paradox in our observations: well-fed young colonies added one hydranth per day. The internodes or distances between adjacent hydranths are (almost exactly) one mm. long. A stem 35 days old should be expected to have 35 hydranths and be 35 mm. tall. Yet our older colonies in the laboratory had only 20 hydranths at the most, and were only about 20 mm. high. Likewise, in nature, stems of this species are rarely as tall as 30 mm. and usually are much shorter.

This discrepancy and the general considerations led to the following questions. Is a slowing down inevitable? Is it gradual or abrupt? Does the ability to grow finally cease entirely? Can experimental procedures be used which eliminate the inhibiting factors? Clear answers should give clues to the nature of such factors.

Figure 1 and the following remarks will enable a reader who is unfamiliar with hydroids to visualize the pattern of growth and understand the terminology employed. A colony has an attached branching stolon system from which stems arise. The first hydranth of a stem, 1 in Figure 1, is produced by upward growth from the stolon. An apical growing zone produces an internode of the main stem, then a pedicel, and finally a hydranth bud. After this there is renewed proliferation from the first internode to produce the next internode, the pedicel of hydranth 2,

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and hydranth 2. This sequence is continued as hydranth after hydranth is added terminally. The increasing height is due not to continuous apical growth but rather to a renewal of proliferative activity by a succession of zones, each of which, at the time of its activity, is in a location just proximal to the most distal hydranth, (GZ in Fig. 1).

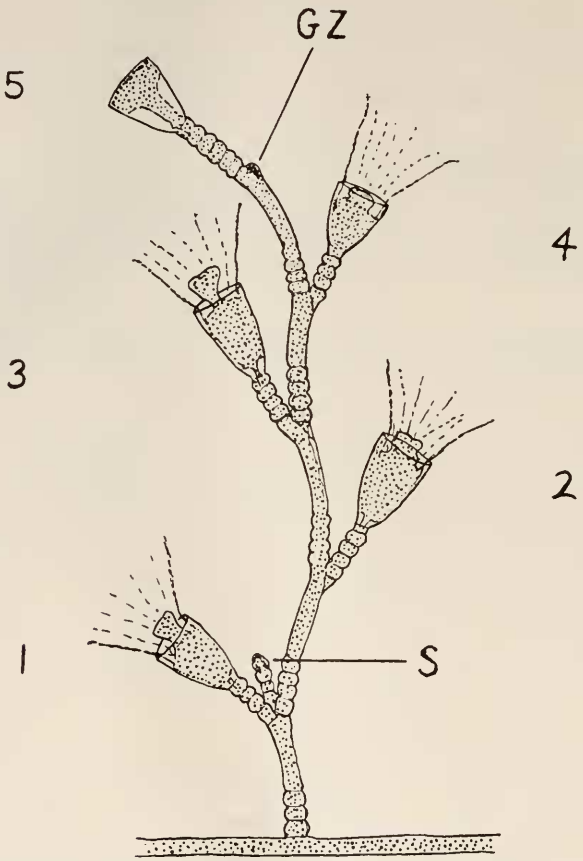

FIGURE 1. A young upright showing the pattern of growth and the location of the terminal growth zone. GZ, terminal growth zone; S, secondary growth which may produce a branch or a gonangium; 1—5 the designations of the nodes and of the primary hydranths or positions.

In *Campanularia* both gonangia and lateral branches develop later from some of the crotches (S in Fig. 1). Also, hydranths live for only about a week, after which they undergo regression and are replaced by a new hydranth at the same location (Crowell, 1953). These secondary developments may be relevant to the problem of apical growth since they must be in competition with the apical growth zone for available nutrition, and perhaps also in less obvious ways. In this report, however, they have been disregarded.

The term *stem* (or *hydrocaulus*), strictly speaking, means only the stem itself. There is no technical term for the stem plus all the hydranths, side branches, and

gonangia which it bears. We call these *uprights:* that which grows up from, or away from, the stolon. An approximate botanical equivalent is *shoot.* The term *position* is used to indicate the primary hydranths or nodes. Each is numbered as in Figure 1. Distances along an upright are described in number of positions.

The colonies used for experimentation were developed from cuttings from cultures originally obtained at Woods Hole two years earlier. These grew on microscope slides, in running filtered sea water cooled to 19° C. Colonies were fed twice a day by placing them in a dense suspension of newly hatched brine shrimp (*Artemia*) for 5–10 minutes, a procedure which provides nutrition for maximal growth.

The Rate of Terminal Growth

Since terminal growth is not continuous, but is a consequence of renewal of activity by tissues just proximal to the last hydranth, the best measure of rate which we have been able to devise is the time in hours from the *cone stage* of the terminal hydranth to the same stage of the next terminal hydranth. The *cone stage* may be thought of as a hydranth bud; it represents a point in the development of a hydranth at which cellular proliferation is about finished and differentiation is commencing. During one three-day period, observations were made every three hours to determine exactly the time difference between each of the developmental stages of a hydranth. With this information it was possible, in the principal experiments, to make observations once each day and then convert these to the time at which the cone had been or would be present. Since this method depends upon the assumption that once development of a hydranth is initiated it develops at a constant rate, the evidence for this needs to be set forth.

In the series of close observations made to establish the normal times for development, we found no significant differences between old and young uprights except in the period of delay prior to the beginning of development. The same is true when specimens at different nutritive levels are compared (Crowell, 1957). Regrettably, the earlier report of Lund (1923) was overlooked in that paper. Lund gives detailed measurements and states (p. 86) that "The sequence in the formation of polyps on isolated internodes of *Obelia* is not due to a difference in rate of regeneration, but is due to a difference in the period of delay between cutting and initiation of the regeneration process. The rate of regeneration (growth) of polyps is the same in basal and apical internodes." *Obelia* is almost identical with *Campanularia* in its growth. An analogous condition is reported by Steinberg (1955) in *Tubularia,* a hydroid quite different from *Campanularia.* He finds that the difference in time of hydranth regeneration under varied conditions is in the preparatory phase, but not in later stages.

In the first experiment several colonies with a large number of uprights were used. Each day a record was made of the stage of development of the terminal hydranth of each upright. Every 2–3 days new stolons were removed so that each colony consisted of only about 20 of the oldest uprights. At the end of 5 weeks this trimming was stopped to permit the growth of a "crop" of young uprights. When the latter had reached a height of about 10 positions, both they and the older uprights were used for the second series of experiments described later on.

On two occasions during the experiment, when the *Artemia* supply was known to be sub-maximal, old uprights which had been adding a new position every

30–35 hours would suddenly require from 72–144 hours. The older the upright, the more pronounced was this slowing down. With equal suddenness, however, as soon as they were returned to maximum feeding, the time shortened to 30–35 hours. Large values obtained during times of food depletion were not included in the averages.

The rate of terminal growth, measured in hours, from cone stage to cone stage, is shown for each position on the uprights in Table I and in Figure 2. Rate, strictly speaking, should be expressed as quantity per unit time, or reciprocal of hours. The conversion seems unnecessary here. A few uprights were kept be-

TABLES I–III

Hours required for the development of a new terminal hydranth at each position on an upright. In each table is shown the position, the number of cases, the mean, and the standard deviation. The mean values are plotted in Figure 2

Table I. The main series of observations; —● in Figure 2.

Position	n	M	SD
1	48	24.9	4.1
2	50	23.8	4.9
3	50	22.1	3.7
4	53	23.6	4.8
5	54	23.8	5.4
6	53	24.5	4.7
7	51	24.4	4.2
8	52	24.9	4.7
9	47	26.2	4.2
10	42	27.3	3.7
11	36	26.1	3.6
12	37	28.6	4.0
13	42	29.2	4.2
14	43	30.0	5.7
15	45	30.8	4.5
16	39	30.8	4.9
17	31	31.3	4.3
18	33	32.4	5.5
19	38	33.4	6.0
20	35	35.6	5.1
21	39	35.6	5.8
22	36	32.0	4.9
23	41	33.8	6.6
24	47	32.4	4.5
25	49	32.6	6.0
26	48	33.9	7.7
27	45	32.8	10.7
28	36	32.9	5.8
29	28	31.6	6.1

Table II. The old controls (0:con) during the experimental period; —○ in Figure 2.

Position	n	M	SD
31	10	28.6	3.5
32	10	32.0	4.4
33	10	30.0	3.1
34	9	32.6	4.9
35	8	30.5	4.0
36	7	32.9	2.7
37	6	31.8	4.4
38	4	32.0	2.5

Table III. Young controls (N:con) before and during the experimental period; —× in Figure 2.

Position	n	M	SD
1	12	27.3	5.3
2	17	23.4	4.9
3	18	21.6	3.4
4	18	21.7	4.1
5	18	22.5	3.1
6	18	22.0	2.0
7	18	22.4	3.4
8	18	22.4	2.4
9	18	23.3	3.0
10	17	22.9	2.0
11	18	25.4	3.7
12	18	25.1	2.8
13	17	27.1	3.9
14	18	29.1	4.5
15	17	29.0	5.4
16	16	28.6	3.3
17	16	30.1	3.8
18	12	30.1	4.4

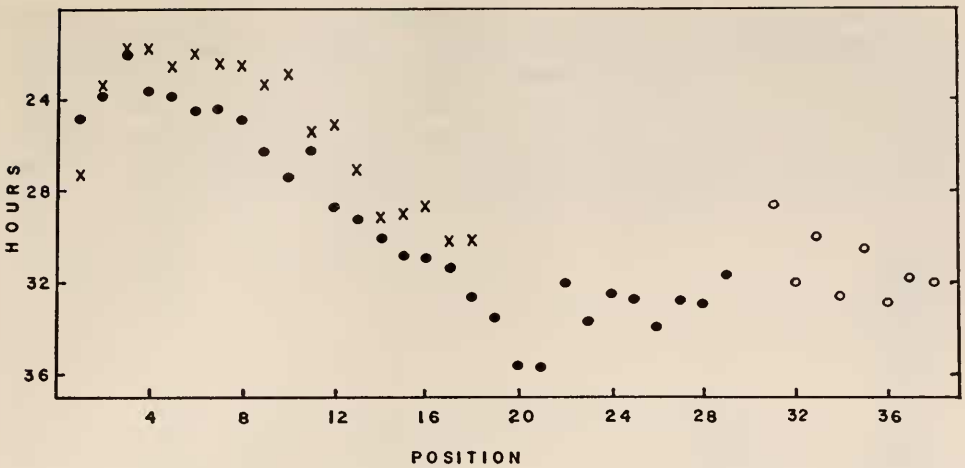

FIGURE 2. The rate of development of terminal hydranths, expressed as number of hours from one cone stage to the next, for each position on a stem. ●, main series of observations. ○, a few of the same stems continued (0:con). X—a second series of observations (N:con). Data in Tables I–III.

yond the 30th position and used as controls in the subsequent experiments. These are plotted as 0 in Figure 2 and the data are given in Table II. Also, a second series of values was available for young uprights and these are shown by X in Figure 2 and are presented as Table III. The slightly higher values obtained in these later observations are probably a reflection of some slight, but unrecognized, improvement in conditions. In Figure 2 three periods are identifiable: During the first 8–10 days one new hydranth is added each 24 hours; then the rate becomes slower until about 20 positions are established; after this there is a period of steady rate at about 33 hours. How long this might continue no one knows. The oldest stems, at about 60 days, were taller than any we have seen either in nature or in our ordinary cultures.

AMPUTATED UPRIGHTS AND SECTIONS OF UPRIGHTS

The following experiments were designed to show the terminal growth rate when the relationship of the terminal growth zone to the rest of the upright and to the colony is altered. The uprights whose history had been closely followed for 5 weeks, and the newer uprights which were 8–13 positions high, were cut according to the scheme shown in Table IV and Figure 3. Those sections which were detached were held in place at the edge of slides by a thread. Their proximal ends, as well as the basal ends of any lateral branches, were ligatured with a single strand of Dacron to prevent growth at all but the distal end.

Measurements were made for the time from cone stage to cone stage for the four hydranths produced terminally on each upright of the control groups. In the experimental groups the hydranth develops from the cut end of the upright and the time is measured from the moment of cutting to the cone stage. The times for the three successive hydranths, which develop from the zone of prospective growth, are measured as before, from cone stage to cone stage.

In these experiments the distal cut end sometimes failed to produce a hydranth, but instead produced an indeterminate growth similar to a stolon. These are called *free* (*i.e.,* not attached) *stolons.* When they developed we cut them off. Following such amputation, a free stolon sometimes developed again and sometimes the new growth was a hydranth. This complicates the analysis and makes it necessary to report both the rate of hydranth development when it did occur and also the incidence of hydranth development as compared with that of free stolons.

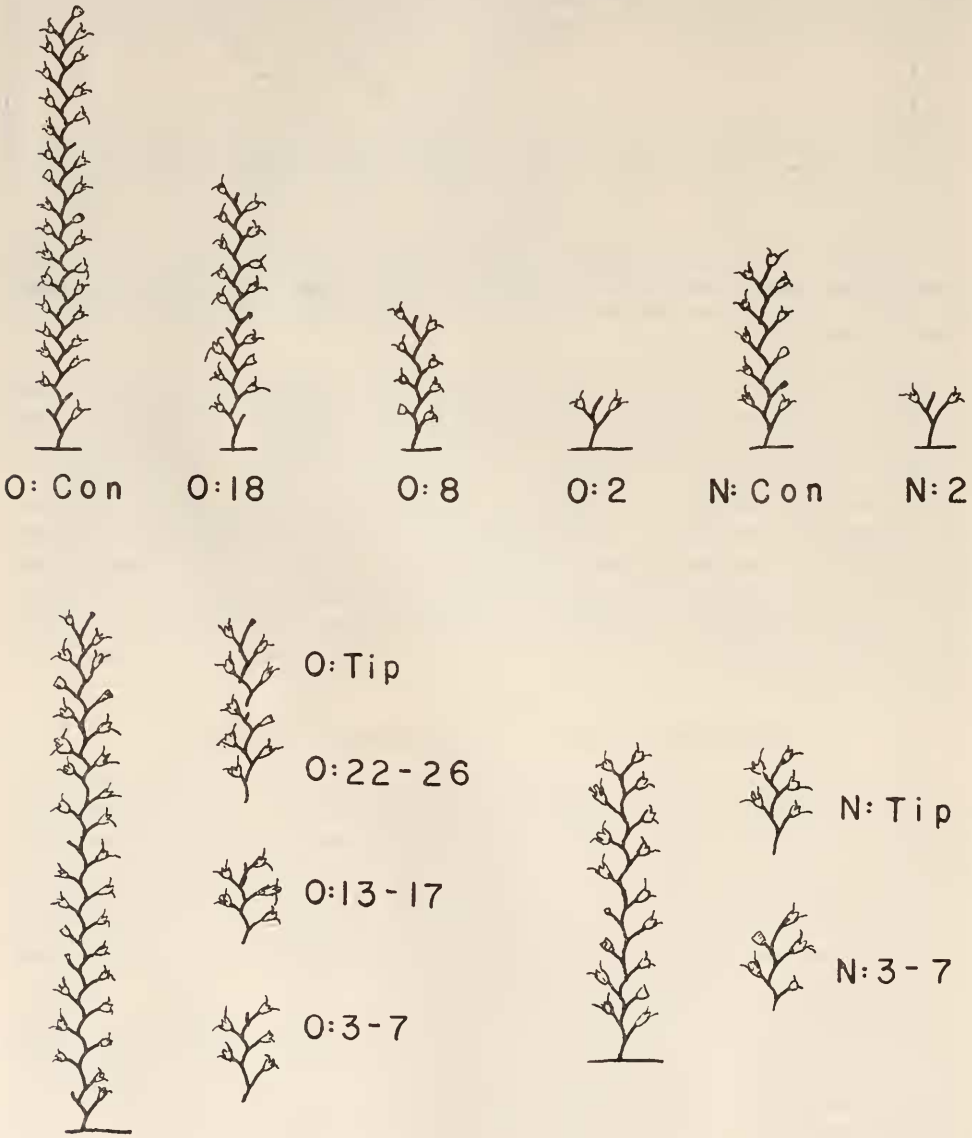

FIGURE 3. Diagrams to illustrate the plan of the experiments as outlined in Table IV.

TABLE IV

Plan of experiment, shown also in Figure 3. The abbreviations at the left are the designations given the experimental groups

From older series of uprights, 0:

0:con	Controls with about 30 positions, connected with rest of colony
0:18	Trimmed to leave 18 positions, connected with rest of colony
0:8	Trimmed to leave 8 positions, connected with rest of colony
0:2	Trimmed to leave 2 positions, connected with rest of colony
0:3–7	Sections consisting of positions 3 to 7
0:13–17	Sections consisting of positions 13 to 17
0:22–26	Sections consisting of positions 22 to 26
0:tip	Sections with 5 positions including the uninjured terminal position (approximately 32–35)

From younger series of uprights, N:

N:con	Controls with about 10 positions, connected with rest of colony
N:2	Trimmed to leave 2 positions, connected with rest of colony
N:3–7	Sections consisting of positions 3–7
N:tip	Sections with 5 positions including the uninjured terminal position (approximately 12–15)

Since the development of a hydranth involves more differentiation and organization than the production of a free stolon, we think of the former as more "difficult" as well as more effective in respect to the normal growth of the colony.

Both in describing the results and in the discussion the word *significantly* has been used only when differences are significant at the 1% level as determined by "t" test.

The data are presented in Table V, A. The young controls, N:con, have a value of 26.6 hours for positions 12–15. This is significantly faster than the rate for the old controls at 30.7 hours. The old uprights cut near the base, 0:2 and 0:8, have rates well above 40 hours. They are significantly slower than old and young controls and also slower than the young uprights, N:2, similarly amputated. The poorest rate is seen for the few uprights which produced hydranths when cut at the nineteenth internode, 0:18: 56.4 hours (significantly slower at the 5% level than 0:2, in spite of the small number of cases).

The times for the development of the first hydranth are shown in Table V, A in a separate column. Since once development starts, its rate is the same for all groups, differences among them must be a reflection of the time required for healing and preparation for proliferation. The O: – groups are nearly identical one with another but slower than the younger N:2 group.

Table V, B shows the percentages of cases in which the trimmed uprights produced hydranths rather than free stolons at the cut surface. In one column is shown the percentage of cases in which hydranths were produced at the first opportunity, in the last column the percentage of cases which finally produced a hydranth after one or more removals of the stolon which had grown at first. The groups stand in the same relationship one to another whichever column is used. Comparison of the values among the groups in Table V, B with those of Table V, A shows that the uprights which produce hydranths most rapidly are the same ones which more often produce hydranths rather than free stolons.

In the "effectiveness" or "efficiency" of terminal growth the experimental groups

stand in the following order: Tips of unamputated young uprights — N : con; tips of unamputated old uprights — O : con; young uprights amputated near their base — N:2; old uprights amputated at the third or ninth internode — 0:2 and 0:8; and old uprights amputated at the nineteenth internode — 0:18.

TABLE V

The development of hydranths and free stolons from amputated uprights

Experimental group	Position of new hydranths	Hours for hydranth production			Hydranths produced instead of free stolons		
		Number of cases	Cone to cone	Cut to cone	Number of cases	% the first time	% at any time
Trimmed Uprights							
		A			B		
0:2	3 4–6	14 38	43.9 ± 12.2	37.9 ± 6.0	31	16	74
0:8	9 10–12	9 25	47.6 ± 12.8	35.9 ± 4.9	16	19	75
0:18	19 20–22	7 5	56.4 ± 20.3	37.1 ± 5.4	12	8	58
0:con	32–35	39	30.7 ± 4.2				
N:2	3 4–6	26 69	35.2 ± 8.1	32.5 ± 2.3	33	52	97
N:con	12–15	71	26.6 ± 4.0				
Isolated Sections							
		C			D		
0:3–7	8 9–11	9 21	43.9 ± 12.4	29.3 ± 7.1	19	16	32
0:13–17	18 19–21	4 11	44.1 ± 10.1	33.8 ± 7.1	15	13	27
0:22–26	27 28–30	9 26	36.2 ± 6.1	30.2 ± 4.2	16	56	68
0:tip	32–35	45	35.6 ± 8.3				
N:3–7	8 9–11	17 48	43.0 ± 12.4	32.4 ± 7.1	19	74	95
N:tip	12–15	60	36.0 ± 9.2				

Before considering the results of the measurements on the isolated sections of uprights, it is necessary to point out that the values for them cannot be directly compared with those of the specimens still attached to the rest of the colony. Because there was a good deal of regression of hydranths in segments isolated from lower levels of the uprights and because it seemed essential to be able to compare the group one with another, we fed each specimen two brine shrimp a day for each position present (whether a hydranth was at each position or not). This gives as constant a nutritional intake as can be achieved. It is at a level known to be adequate for moderate, but not maximal, growth (Crowell, 1957).

As before, the times are calculated separately for the first hydranth to be produced (from the time of cutting) and for the subsequent three. Data are presented in Table V, C and D.

The time required for the development of the first hydranth is nearly equal for the four groups.

The growing tips of new and old colonies are almost identical in rate although they are at levels 20 positions apart. That their rates are somewhat slower than those of intact uprights is interpreted as due to the lower quantity of food received (discussed above). Levels farther from the growing tips have slower rates. The level nearest the tip, 0: 22–26, is essentially equal to the tip, and significantly better than the lower two levels, 0: 13–17 and 0: 3–7 which are alike. N: 3–7, even though it came from a young upright, and was only about 7 positions below the growing tip, is also slow.

In the case of the amputated uprights it will be recalled that there was a close parallelism between rate of hydranth production and the tendency to produce hydranths rather than free stolons. The same is true for the isolated sections (Table V, D), with one exception: the N: 3–7 group had a slow rate but rarely produced stolons.

Discussion and Conclusions

The continuous record for 60 days of the terminal growth of uprights (Fig. 2) shows that when nutrition is optimal, the growth rate is maximal for about 10 days, then gradually reaches a new and lower level which it maintains thereafter. One can postulate that whatever factors reduce the rate from about 24 to 33 hours, these have no further effect, and, at the somewhat slower rate, terminal growth could continue forever. In nature a long stem would, of course, eventually be broken off. In the laboratory one could cut off the distal portion and follow its history, an experiment which we have not yet carried out. The "immortal" hydras described by Brien and Reniers-DeCoen (1949), and the successful indefinite asexual reproduction of some oligochaetes and turbellarians (*e.g. Stenostomum*, Sonneborn, 1930) are examples of growth without limitation somewhat comparable to the situation described here.

Earlier experiments (Crowell, 1957) have shown that the height or age of an upright determines quite precisely the extent to which lowered nutritive level affects the rate of terminal growth. This effect of age (or height) is evident in stems when they are only a few positions high or a few days old and indicates that at least one factor which can influence growth is accumulating long before it can become effective in well-fed colonies.

The experiments involving the trimming of uprights and isolation of sections at different levels were designed with some prejudice in favor of a correlation between the rate of terminal growth and the age of the tissues. Although the terminal growth zone is always young in actively growing colonies, it is still possible that there occurs an inherent slowing down with time, independent of factors external to the terminal growth region itself. In the absence of this condition it may be possible that the older tissues below the terminal zone, as they become older, are the source of factors which adversely affect terminal growth.

Analysis of the data has made it necessary to consider four possibilities. 1) Correlation with age of tissues as discussed above. 2) Inhibitory substances arising from hydranths already present, but not correlated with the age itself of these hydranths—perhaps a process of like inhibiting like. 3) Deficiency of nutrition as the distance from the stolon becomes greater. This might merely be a consequence of less efficient hydroplasmic streaming in the distal portion of tall uprights. 4) Any combination of the above.

The factors suggested are not the only possible ones. Hydranth regression, production of lateral branches and the development of gonangia may have effects, but this question has not yet been examined. There are, of course, still other possibilities.

If slowing of terminal growth were inherent to the growing terminal portion of the upright, one would not expect this to be expressed during only the production of the tenth to twentieth positions. Tips of old and young uprights, different in length by 20 positions, when isolated showed the same rates of terminal growth (0 : tip and N : tip in Table V, C).

When we compare groups 0 : 3–7, 0 : 13–17, 0 : 22–26, and 0 : tip, the similarity of rate between the latter two suggests that no marked slowing has occurred 7 positions below the tip. However, those about 15 positions below the tip, 0 : 13–17, show a significantly slower rate. The oldest group, 0 : 3–7, is the same as 0 : 13–17. It should be emphasized that each of these specimens received the same amount of food; hence differences cannot be accounted for on the basis of nutritional intake. Further evidence of a correlation with age is seen in the comparison of groups N : 3–7 and N : tip. The former, comprising tissue about 7 positions back from the tip, shows a significantly slower rate than the tip. Comparisons in Table V, A between N : 2 and N : con and between N : 2 and 0 : 2 also show a correspondence between rate and age of tissues.

It is evident that there is no aging at the growing tip itself. It is also clear that the rate of hydranth formation at increasing distances from the tip and in older tissues is slower. The tissues do not express the consequence of aging until about 7 hydranths have been produced beyond them, that is, about 10 days after the tissue had been first established. At this time this effect increases sharply; soon it becomes maximal and thereafter there is no further decrease in rate related to the increasing age of the tissues. The similar rates shown by 0 : 3–7 and 0 : 13–17 support this last statement.

Not all of the results are consistent with the idea that aging effects explain the slowing of terminal growth. The experiments do not distinguish between the role of possible inhibitors and that of the efficiency of circulation. With increasing length of upright there is not only an increase in the number of hydranths to produce an inhibition, but also a greater length of coenosarc separating the proliferat-

ing zone from the basal stolon. The chief indication that one or both of these factors are operating is seen in Table V, A, particularly for the old uprights. With increasing distance from the stolon (decreasing age of tissues) there is an increase in the time required for hydranth production. Statistically, 0:2 with the faster rate is not significantly different from 0:8, but is different at the 5% level from 0:18. These results are the opposite to those expected as a consequence of aging and indicate that some factor(s) exert an effect opposite to that of aging.

The frequency with which the cut specimens developed free stolons was higher the slower the rate of hydranth production. To this generalization there was one exception: the N:3–7 group which produced relatively few free stolons but had a slow rate for hydranth production. This same group has been mentioned earlier in connection with the effect of age of the tissues. It had a slower rate than tissue of about the same age belonging to older uprights, the 0:22–26 group. We are unable to decide whether we should regard this case as anomalous in respect to its rate or in respect to the frequency of free stolon production. Its significance may lie in the hint which it gives that the factors which influence the production of free stolons may be different from those which control rate.

One result, so far mentioned only incidentally, is shown in Table V, A and C. For those cases in which the distal tip had been severed, the rate of replacement of the first hydranth, that is the one arising from the cut surface, is tabulated separately from the rates for successive hydranths. For all of the groups there is little difference in the rates at the cut surface but marked differences for hydranths subsequently produced. Lund (1923) found differences in replacement of hydranths of *Obelia* correlated with the distance from the growing tip. The absence of such differences in our specimens is unexpected.

Although the rates for the production of the hydranth from the cut surface are similar for all our cases there was great variation in the frequency of free stolon production by these same cut ends. Apparently if a hydranth is to develop, it can begin to do so with about the same speed regardless of the level of the cut or age of tissue. A correlation with age is seen in the frequency of free stolon production, but the effect of age applies to hydranth production only after the first has been produced.

The general conclusion from these many considerations and comparisons is that there is a period of maximal rate of terminal growth expressed by uprights only during their first 10 days; after this the zones of prospective terminal growth become adversely affected by factors external to themselves. Although the age of cut sections of stems is one factor correlated with slower hydranth production and with the development of free stolons instead of hydranths, many of the results cannot be explained on this basis. What these other factors may be can only be conjectured until further experiments are carried out.

Summary

1. When terminal growth rate was measured for stems of well-fed colonies of the hydroid *Campanularia flexuosa* for a period of 60 days, it was found that this rate is constant for about ten days, becomes progressively slower for the next 10 to 15 days, and then remains constant.

2. Isolated sections of stems of different ages differ one from another both in

respect to the time required for the production of additional terminal hydranths, and in their ability to produce hydranths rather than free stolons. In general the same experimental groups which most readily produce hydranths following cutting produce them at fairly high rates.

3. Older levels of stems are in general less efficient in the rate of terminal hydranth production and in the ability to produce hydranths rather than free stolons. Not all of the results can be explained on the basis of an effect of aging. The possible role of inhibitors and of differences in efficiency of circulation must be considered.

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